ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Magnitude and Antimicrobial Resistance Pattern of *Staphylococcus aureus* among Mother Child pairs isolated from nasal swab at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

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A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology)

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Approved by the Examining Board

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External Examiner	**Signature Date**

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Abbreviations

AMP	Antimicrobial Peptides
AMR	Antimicrobial Resistance
AST	Antimicrobial Susceptibility Test
CA MRSA	Community Acquired Methicillin-Resistant *Staphylococcus aureus*
DCC	Day Care Center
EOPD	Emergency out Patient Department
EPI	Expanded Program on immunization
ESBL	Extended Spectrum B-Lactamase
HA MRSA	Hospital Acquired Methicillin-Resistant *Staphylococcus aureus*
HCW	Health Care Workers
LA MRSA	Livestock Associated Methicillin-Resistant *Staphylococcus aureus*
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NICU	Neonatal Intensive Care Unit
PBP-2a	Penicillin Binding Protein-2a
PMN	Polymorphonuclear Cells
SCCmec	Staphylococcal Cassette Chromosome
SPSS	Stastical Package for the Social Sciences.
SSI	Skin and Soft Tissue Infection
TASH	Tikur Anbessa Specialized Hospital
VISA	vancomycin intermediate *Staphylococcus aureus*
VRSA	Vancomycin-resistant *Staphylococcus aureus*

Abstract

Background: *Staphylococcus aureus* is a leading cause of hospital acquired infections. It's the primary cause of lower respiratory tract infections, surgical site infections, second leading cause of nosocomial bacteremia and cardiovascular infection. Methicillin-Resistant *Staphylococcus aureus* infections in the pediatric population are associated with significant morbidity and hospital costs. Mode of transmission from mother to child by shortly after birth and can become colonized quickly after contact with adult skin or their environment.

Objectives: To determine the Magnitude and antimicrobial resistance pattern of *Staphylococcus aureus* among mother child pair from nasal swab at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Methods: A cross-sectional study were conducted from March to July 2018 among pediatric emergency outpatients and expanded program on Immunization aged under five in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. Nasal swab were collected using sterilized cotton swab from each child and mother. Standard techniques of culture on both blood agar and Mannitol Salt agar were used for isolation and identification of *S. aureus* along with catalase and coagulase test .Antimicrobial susceptibility test was performed using a disk diffusion method on Muller Hinton agar. The data were analyzed by stastical package for the social sciences package software version 20.0 and Microsoft excel.

Results: From total of 212 mother child pair 25(11.8%) child and 31(14.6) mothers had *Staphylococcus aureus* in their nasal cavity. The mean age of pediatrics participants in this study was 2.0047 ± 0.58 (SD standard deviation) years. Two Methicillin-Resistant *Staphylococcus aureus* isolates was detected from mothers in pediatrics EOPD 2(3.6%). About 89.3% of *Staphylococcus aureus* was resistance to Penicillin and 17.9% which were also resistance to Gentamicin (Tobramycin).

Conclusion: This study showed lower rate of nasal carriage of mother child pair of *S.aureus* and Methicillin-Resistant Staphylococcus aureus. Therefore developing decolonization protocols and proper utilization of drugs are needed to reduce the transmission of *S.aureus* and the burden of Methicillin-Resistant *Staphylococcus aureus.*

Key Words: MRSA, Mother Child pair, AST, TASH

1 Introduction

1.1 Background

Staphylococci are Gram-positive cocci 1μm in diameter. They form clumps and they are non-motile [1].*Staphylococcus aureus* is a leading cause of hospital-acquired infections. It is the primary cause of lower respiratory tract infections, surgical site infections, second leading cause of nosocomial bacteremia and cardiovascular infections [2].

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged as a clinically relevant human pathogen more than 5 decades ago. The virulent bacterium was first detected in hospitals and other health care facilities where vulnerable hosts, frequent exposure to the selective pressure of intensive antimicrobial therapy, and the necessity for invasive procedures created a favorable environment for dissemination. MRSA emerged as an important cause of health care-acquired infections, particularly central line-associated bloodstream infection, ventilator-associated pneumonia, and surgical site infection [3].

MRSA is common in the pediatric population and associated with significant morbidity and hospital costs [4].

In addition, children may act as vectors for spreading *S. aureus* and MRSA to both community and hospital environments. Several determinants of *S. aureus* carriage in healthy children have been suggested, examples of which are the number of siblings, family size, and day care center (DCC) attendance. DCCs constitute reservoirs of MRSA where children are at increased risk of nasal colonization by *S. aureus* [5].

Community acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) strains affect a population distinct from those affected by Hospital acquired Methicillin-Resistant *Staphylococcus aureus* (HA-MRSA) and cause different clinical syndromes. CA-MRSA infections tend to occur in previously healthy younger patients. It has been associated predominantly with skin and soft tissue infection but has also been linked to several severe clinical syndromes such as necrotizing pneumonia and severe sepsis. In contrast, HA-MRSA strains have been isolated largely from people who are exposed to the health care setting; the

patients are older and have one or more comorbid conditions. HA-MRSA strains tend to cause pneumonia, bacteremia, and invasive infections [6].

Neonates are exposed to *S.aureus* shortly after birth and can become colonized quickly after contact with adult skin or their environment. Between 30–70% of humans are carriers of *S.aureus*, so neonates have a very high likelihood of exposure during the immediate period after birth. For MRSA, the nares and umbilicus are the most common sites of initial colonization.

Additional factors, such as overcrowding and understaffing in the NICU, have been associated with increased risk of healthcare-associated transmission and colonization, which may lead to epidemics of MRSA infection [7].

S.aureus is naturally susceptible to virtually every antibiotic that has ever been developed. Resistance is often acquired by horizontal transfer to genes from outside sources, although chromosomal mutation and antibiotic selection are also important. This exquisite susceptibility of *S. aureus* led to Alexander Fleming's discovery of penicillin, ushering in the "antibiotic era." Penicillin was truly a miracle drug: Uniformly fatal infections could be cured. Yet, by the mid-1940s, only a few years after its introduction into clinical practice, penicillin resistance was encountered in hospitals and within a decade it had become a significant problem in the community. *S. aureus* is remarkable in its ability to acquire resistance to any antibiotic [8, 9].

The mecA gene is carried by a mobile genetic element designated staphylococcal cassette chromosome (SCCmec). The SCCmec typing is strong evidence of the independent evolution of HA-MRSA and CA-MRSA clones; SCCmec types I, II, and III are predominantly observed in HA-MRSA strains, while SCCmec types IV and V are primarily found in the CA-MRSA strains. However, it cannot be overemphasized that it is much more than simply the resistance to methicillin that defines the virulence of CA-MRSA [10].

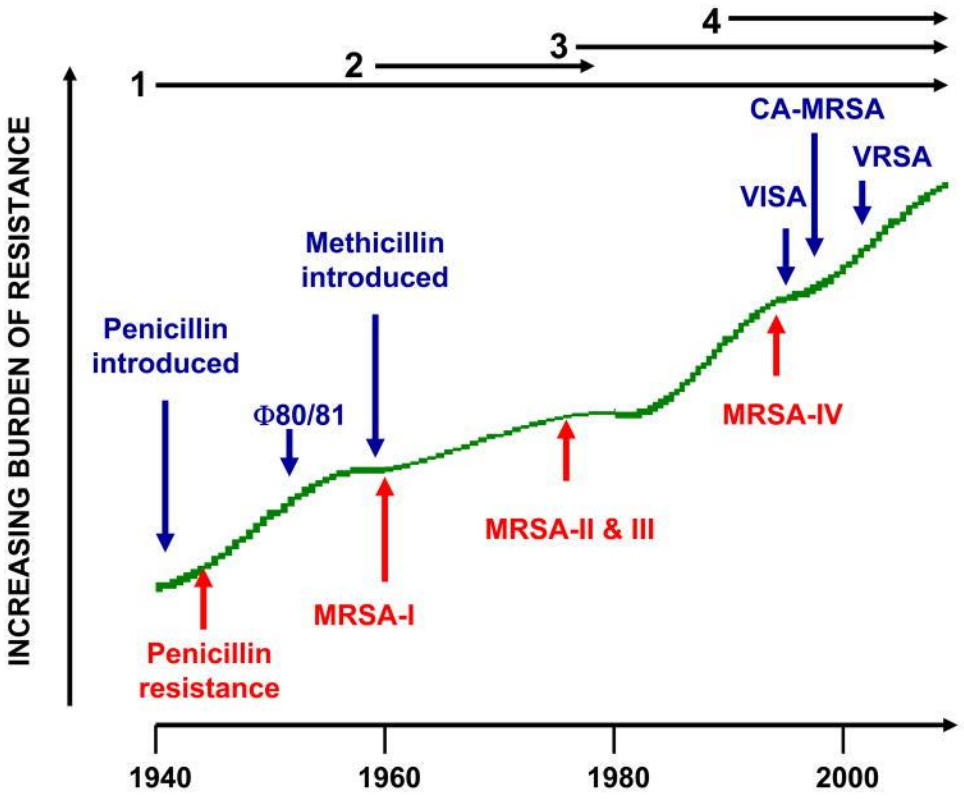

Figure 1: A timeline of the four waves of antibiotic resistance in *S.aureus* [11].

Mechanisms of resistance, methicillin resistance requires the presence of the chromosomally localized mecA gene [12, 13]. mecA is responsible for synthesis of penicillin-binding protein 2a (PBP2a; also called PBP2′) a 78-kDa protein. PBPs are membrane-bound enzymes that catalyze the trans-peptidation reaction that is necessary for cross-linkage of peptidoglycan chains. Their activity is similar to that of serine proteases, from which they appear to have evolved. PBP2a substitutes for the other PBPs and because of its low affinity for all β-lactam antibiotics, enables *staphylococci* to survive exposure to high concentrations of these agents. Thus, resistance to methicillin confers resistance to all β-lactam agents, including cephalosporins. Recent studies determined the crystal structure of a soluble derivative of PBP2a. PBP2a differs from other PBPs in that its active site blocks binding of all β-lactams but allows the transpeptidation reaction to proceed [14].

1.2 Statement of the problem

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes ~100 000 invasive infections and ~20 000 deaths per year in the United States; of these, ~1000 infections and ~100 deaths occur in children <1 year of age. MRSA causes between 59% and 72% of all skin, soft-tissue infections (SSTIs) and up to 95% of all SSTIs in children are caused by CA-MRSA. MRSA now affects previously healthy individuals without known risk factors. CA-MRSA has become the most frequent clone of *S. aureus* in many communities, causing disease in neonatal intensive care units (NICUs) and even among healthy full-term babies [15].

According to World Health Organization report showed that S.aureus are the most commonly reported resistant bacteria and MRSA are fifth greatest public health problem[16].

The treatment of MRSA infection is challenging because of their high resistance to different antibiotics [17].

Although epidemiological data from separate studies are often not comparable owing to differences in study design and populations sampled, the highest rates (>50%) are reported in North, South America, Asia and Malta. Intermediate rates (25–50%) are reported in China, Australia, Africa and some European countries (Portugal (49%), Greece (40%), Italy (37%) and Romania (34%). Other European countries have generally low prevalence rates (Netherlands and Scandinavia) [18].

In low-income countries the incidence of *S. aureus* disease is highest in neonates and children up to one year of age and mortality rates are estimated to be up to 50% [19].

In Ethiopia, MRSA is increasingly reported in hospitals starting from the first report since 1987.According to Geyid et al, the isolation rate of MRSA was 30%. In 1993 a study by Tenssay et al, in Addis Abeba, 40% of the *S.aureus* isolate were found to be MRSA.

MRSA infections are a challenge for physicians in developing countries to treat because of the limited choice of therapeutic options available and due to the possibility of concomitant drug resistance of the MRSA to other antimicrobials. MRSA are also a challenge to patients in developing settings due to increased cost of care. The financial burden of MRSA care in regions of limited resource, such as Ethiopia, is not expected to be easy because there are other priorities such as TB, HIV and malaria [20].

To the best of our knowledge, in Ethiopia there are sufficient data on *S.aureus*, but no published information is available on mother child pair, hence this study attempted to assess weather mother child pair had similar carriage status on *S.aureus* or not.

1.3 Significance of the Study

In Ethiopia there is no updated epidemiological data on *S.aureus* among mother child pair. Hence investigating and monitoring trends in antimicrobial resistance are essential for adequate antimicrobial therapy.

The result of this study will also serve as source of information for;

- Clinicians,
- Policy makers,
- Patients and
- Other interested researchers in this area that can be used for further investigation.

2. Literature Review

2.1 Basic Characteristics and Identification

On bacteriological media, *S.aureus* appears as a large white to golden colony. The majority of the time the colony of *S.aureus* produces a zone of hemolysis surrounding the colony. It is not very fastidious and grows well, either aerobically or under anaerobic conditions and produces good growth within 24 hours. All *Staphylococci* produce the enzyme catalase when introduced to hydrogen peroxide and easily differentiates the *Staphylococci* from the *Streptococci*. It also produces the enzyme coagulase which allows the organism to produce a clot in rabbit plasma. This is a key test to differentiate *S. aureus* from other *Staphylococci* [21].

2.2 Carriage of *S.aureus* and MRSA

HCWs, patients and the environment may act as reservoirs for the spread of MRSA to patients and others. Screening and eradication of MRSA colonization is an effective method of reducing the MRSA infection rate [22]. *S.aureus* is carried by 20–30% of the human population, defined as persistent carriers, whereas the remaining part of the population does not carry or is only transiently colonized by this opportunistic pathogen. Colonization of the anterior nares is a recognized risk factor for subsequent *S. aureus* infection, although carriers generally have a better prognosis. Risk factors for colonization include young age, male sex, underlying comorbidities, hospitalization and exposure to livestock [23].

A Cohort study conducted in Greece, 128 newborn infants and their mother, were enrolled. The carriage rate was 43.6% in infants and 42.5% in mothers. Carriage rates were higher in infants with carrier mothers, non-smoking mothers, and many siblings [24].

In China Shenzhen, the prevalence of *S. aureus* and MRSA among mothers was as follows nasal, 25.8%; vaginal, 7.3%; and nasal and vaginal, 3.3% and nasal, 5.7%; vaginal, 1.7%; and nasal and vaginal, 0.5% respectively. The incidences of *S. aureus* and MRSA carriage among neonates were 3.3% and 0.8%, respectively [25].

A study conducted in Gabonese mother–infant pairs was assessed at delivery and after 1, 9 and 12 months. Based on spa typing, direct mother-to-infant transmission was evident in 5.6%. Maternal colonization was a risk factor for *S. aureus* carriage in infants [26].

High carriage rates have been reported from several countries such as in Washington of mothers with MRSA nasal colonization, 45% of their infants had nasal MRSA colonization at 2 months [27], in Nepal 56.1% [28] and low carriage rate also reported in USA, Florida were only 2.1% of mothers and 0.7% of infants carried MRSA [29], in Tanzania 10.5% [30], in Dessie 12.7% [31], and Jimma 18.8% [32].

2.3 Pathogenesis

Infection caused by *S. aureus* can be endogenous, where the infectious organism is found in the patient's body, or exogenous, where the organism is transmitted from an external source [33].

S.aureus causes both local and serious systemic infections in otherwise healthy full-term neonates and infants [34].

The anterior nares are the main ecological niche for *S. aureus*. Approximately 20% of individuals are persistently colonized with *S. aureus*, and 30% are intermittently colonized. However, numerous other sites may be colonized, including the axillae, groin, and gastrointestinal tract [35].

S.aureus causes a wide range of infectious syndromes, Primary skin infection impetigo, which is transmitted from person to person, secondary skin infections associated with eczema, surgical wounds, intravenous devices, burns, Pneumonia rare, but may follow influenza and progress rapidly with cavity formation, endocarditis can be rapid and destructive; associated with intravenous drug misuse or colonization of intravenous devices, osteomyelitis and Septic arthritis [36].

Abscesses are a frequent manifestation of *S. aureus* skin and soft tissue infections and are formed, in part, to contain the nidus of infection. Polymorphonuclear leukocytes (neutrophils) are the primary cellular host defense against *S. aureus* infections and a major component of *S. aureus* abscesses [37].

In general, the ability of bacteria to cause disease in humans is due to evasion of innate host defense, which includes resistance to antimicrobial peptides (AMPs) and killing by phagocytic leukocytes. In as much as PMNs (also called neutrophils or granulocytes) constitute the greatest number of leukocytes in humans, they are the primary cellular defense against *S. aureus* infections [38].

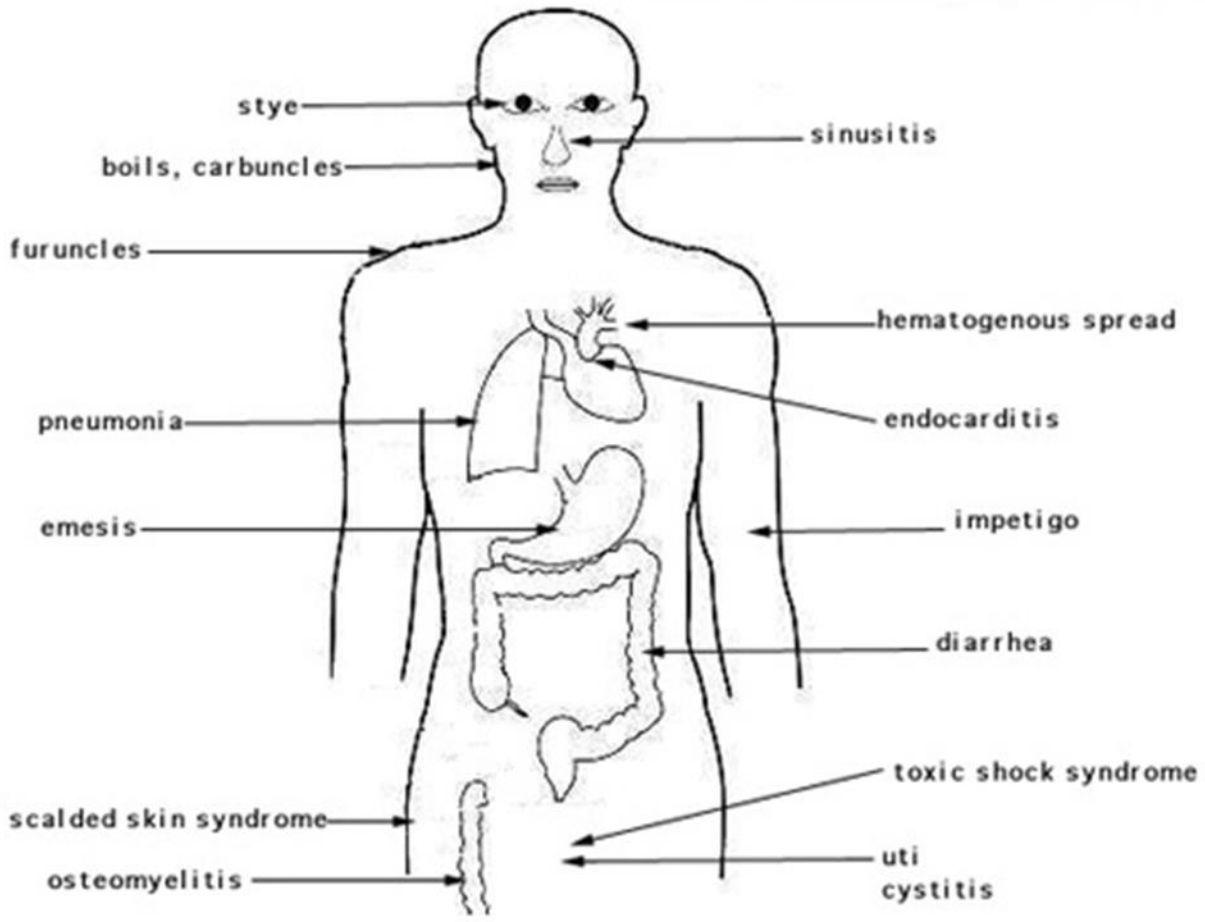

Figure 2; Sites of infection and diseases caused by *S.aureus* [39]

2.4 Diagnosis

There are different kinds of *S.aureus* tests and the simplest and most common one is the culture test. Newer DNA based tests that are faster than culture tests can even produce results in a few hours. A culture test identifies what specific type of bacteria is causing your infection. Coagulase test is used to identify *S.aureus* which produces the enzyme coagulase. Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of *S.aureus*; free coagulase which converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by clotting in the tube test. Bound coagulase (clumping factor) which converts fibrinogen directly to fibrin without requiring a coagulase-reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test. Antibiotic susceptibility testing identifies which antibiotic will work best at treating your specific infection. [40-43].

2.5Transmission and Risk Factors

Mode of transmission of *S.aureus* from mother to child through skin-to-skin contact and breast feeding [44]. Risk factors, such as recent hospitalization, surgery, residence in a long-term care facility, receipt of dialysis, or presence of invasive medical devices [45].

Age of less than four years, male sex, family size being more than four, antibiotic use and admission during the previous three months, parental smoking and sleeping with parents were independent risk factors for nasal colonization with MRSA [46].

2.6Treatment and Prevention

MRSA strains harbor resistance to the beta-lactam antimicrobials including oxacilin, dicloxacilin, cloxacillin, nafcillin, methicillin, and first, second and third generation cephalosporins.

HA-MRSA strains are typically resistant to macrolides, clindamycin (cleoin), aminoglycosides, quinolones and trimethoprim-sulfamethoxazole (Bactrim).They are sensitive to vancomycin (vancocin), fusidic acid, rifampin (Rifadin) and linezolid(zyvox).

CA-MRSA strains are resistant to macrolides and quinolones but usually sensitive to aminoglycosides such as gentamicin (Garamycin), clindamycin and trimethoprim-sulfamethoxazole.

Empiric antimicrobial therapy should be guided by the clinical presentation and when full sensitivities are available, the antimicrobials may be tailored accordingly [47].

S.aureus is contagious and is passed to skin to skin as well as from inanimate surfaces to the skin. Personal prevention can be achieved by good hand washing. For children, the parents or caregiver can keep any wound clean and covered especially when they are in contact with other people.

Schools and days cares can clean the environment regularly with a good bactericidal spray or wash. Hospitals have specific cleaning routines after dismissal of patients from room or a suite [48]

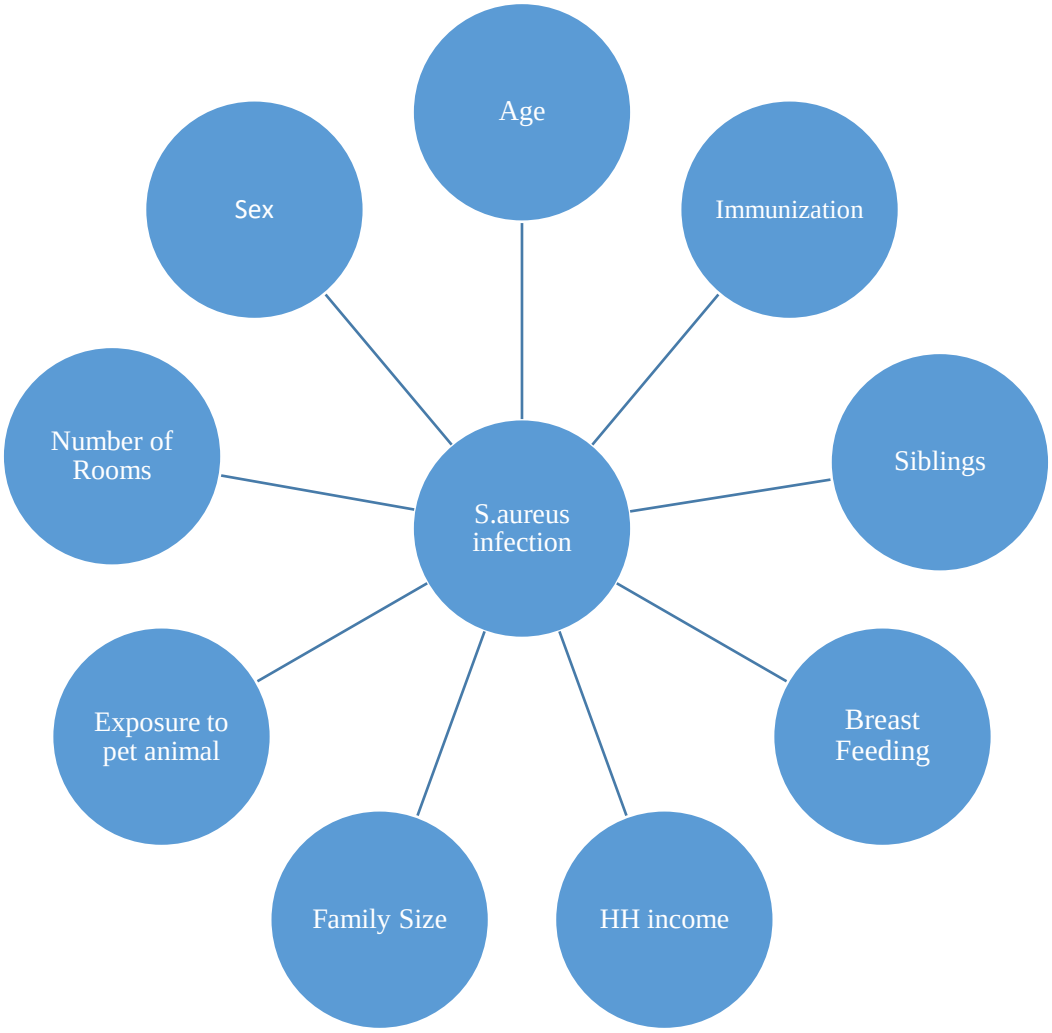

Figure 3. Conceptual frame work for *S.aureus* infection.

3. Objectives

3.1 General objective

To determine the Magnitude and Antimicrobial Resistance Pattern of *S.aureus* among Mother Child pairs isolated from nasal swab at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

3.2 Specific objectives

- ✓ To determine the magnitude of *S.aureus* among mother child under five years of age.
- ✓ To determine antimicrobial resistance pattern of *S.aureus.*

4 Research Question

What is the Magnitude and Antimicrobial Resistance Pattern of *S.aureus* among Mother Child pairs isolated from nasal swab at TASH pediatrics EOPD and EPI department?

5 Material and Methods

5.1Study area

The study was conducted in Tikur Anbessa Specialized Hospital (TASH), the teaching hospital of health Science College, Addis Ababa University. TASH is the largest specialized hospital in Ethiopia, with over 700 beds, and serves as a training center for undergraduate and postgraduate medical students, dentists, nurses, midwives, pharmacists, medical laboratory technologists, radiology technologists, and others who shoulder the health problems of the community and the country at large. With more than 70 percent of childhood deaths attributable to communicable diseases and malnutrition, Ethiopia's healthcare resources have been directed primarily to treat and prevent diseases such as malaria and diarrhea [49]

5.2 Study design and period

A cross-sectional study was conducted from March to July 2018 among mother child pairs of *Staphylococcus aureus* isolated from nasal swab at TASH Addis Ababa, Ethiopia.

5.3Population

5.3.1 Source population

All mother and children under five years of age attending pediatric EOPD and who visits for EPI departments during the study period were eligible for the study.

5.3.2 Study population

All under 5 year's pediatric patients and mothers were visiting the pediatric EOPD and EPI fulfilling the inclusion criterion was the study population.

5.4 Inclusion and Exclusion criteria

5.4 .1 Inclusion criteria

- All Children aged under five years and their mothers attend pediatrics EOPD and EPI departments.
- Those both mother and child volunteer to give nasal sample.

5.4.2 Exclusion criteria

- Children who were critically ill and those taking antibiotics currently within the last 7 days during data collection time were excluded from the study.

5.5Study Variables

5.5.1 Dependent variable

- ✓ Magnitude and antimicrobial resistance pattern of *S.aureus*.

5.5.2 Independent variable

- ✓ Age
- ✓ Sex
- ✓ Brest feeding
- ✓ Currently antibiotics used
- ✓ Sleep with mother
- ✓ History of hospital admission
- ✓ Contact with pet animal
- ✓ Immunization status

5.6 Measurement and Data collection

5.6.1 Sample size calculation

The sample size for the study that infers the total population was determined using a single population proportion formula. There is no prior information about mother child pairs of *S.aureus* in Ethiopia. So I prefer to use p and q 0.5.

n = (Zα)2 (pq)/ d2 Where: n = sample size

Zα/2 = level of confidence

P = MRSA prevalence

q = 0.5 = margin of error (0.05)

$n=z^2 * p * q$, p=0.5, q=0.5, d=0.05, Zα/2=1.96

d^2

(1.96)2*0.5*0.5=385

(0.05)2

Where, N = required sample size for this cross sectional study

Z = Percentiles of the standard normal distribution corresponding to 95% confidence level, which equals to 1.96 (z value at $\alpha = 0.05$)

P = 0.5

d = 0.05 (5% margin of error)

Considering 10% non- response rate = 39 samples were added. Thus a total of 424 children &mothers were enrolled in the study, which means 212 from children and 212 from mothers.

5.6.2Sampling technique

The study subjects were selected using convenient sampling technique from all patients attending TASH among less than five years and their mothers.

5.6.3Data collection procedure

The data was collected by trained nurses from the pediatrics department using well standardized questionnaire and conduct interview mothers of the child to collect the necessary socio demographic information. Socio demographic information includes sex, age, family size, family income, parent's educational background and habits of sleeping with parents.

5.6.4Laboratoryanalysis

5.6.4.1 Nasal swab collection

Nasal swab specimens were collected by trained nurses according to the Centers for Disease Control manual. A specimen was obtained from each child and mother from both anterior nares and placed into amines transport media and processed immediately according to standard procedures.

5.6.4.3 Isolation and Identification of bacteria

The swabs was aseptically inoculated in both blood agar and Mannitol Salt Agar (MSA) and incubated at 35±2°C for 24hrs. After overnight (24 hrs) incubation time golden yellow colonies in the MSA and white creamy colonies in blood agar. For catalase test, resub from blood agar to chocolate agar to avoid false negative and false positive result and gram staining for identification of staphylococci from Streptococci. *S. aureus* identified by coagulase test both slide and tube test method is the golden standard. [50]

5.6.4.4Antibiotic Susceptibility Testing

Modified Kirby-Bauer disc diffusion method

Pure Colonies of isolated bacterial organism was mixed with normal saline and measured at 0.5 McFarland standards for susceptibility testing. The suspensions were streaked onto Mueller Hinton agar plates. The plates were allowed to dry for 3-5 minutes before the disks used. The antibiotic susceptibility testing was performed on the following antibiotic discs; Clindamycin (DA) (2 µg) (lincosamides), Erythromycin (E) (15 µg)(macrolides and ketolides), Penicillin G (P) (10 units)(beta-lactams), Cefoxitin(30 µg)(beta-lactams) , Trimethoprim-sulphamethoxazole (SXT) (1.25/23.75µg)(sulfa drugs), Gentamicin (CN) (10 µg)(aminoglycosides), Tobramycin (10 µg)(aminoglycosides) and Ciprofloxacin (CIP)(5 µg)(quinolones) .The plates was incubated at 35°C ± 2 °C for 24hrs and interpretation made based on CLSI guidelines 2018 [51].

5.6.4.5 Methicillin resistant Staphylococcus aureus (MRSA)

Phenotypic test for Methicillin Resistant (MRSA) and Inducible Clindamycin Resistant (iMLS*B*) *Staphylococcus aureus*. Methicillin resistant *Staphylococcus aureus* (MRSA) isolates were detected by cefoxitin disk (30 μg) method of CLSI 2018. *S. aureus* isolates are deemed methicillin resistant when the zone of inhibition (ZOI) for cefoxitin was ≤21mm. Similarly, inducible macrolide-lincosamide-streptogramin-B (iMLSB) resistance was detected in *S. aureus* by disk approximation using clindamycin (2 μg) and erythromycin (15 μg) on Mueller–Hinton agar plates. After overnight incubation, isolates with flattened zone of inhibition adjacent to the erythromycin disk (referred to as a “D” zone) were considered to exhibit inducible clindamycin resistance.

5.7Data Quality assurances

Pre-Analytical: Media preparation as per manufacturer instruction and laboratory Standard Operating Procedures (SOP) was strictly followed. Verify that media meet expiration date and quality control parameters per CLSI. Labeling container, media, filling the forms were be carried out.

Analytical: Visual inspections of cracks in media or plastic petri-dishes, unequal fill, evidence of freezing, bubbles, and contamination was performed. Use ATCC control strain (ATCC-25923) for isolated *S. aureus.*

Post-Analytical: Report the results on log sheet and store for further data. Samples were stored at -80 0c in skim milk with 15% glycerol.

5.8Data analysis and interpretation

The data was analyzed by SPSS package software version SPSS 20.0 and Microsoft excel. A descriptive analysis was used to determine the demographic characteristics and the magnitude of each isolate.

5.9Ethical Considerations

The study was conducted after ethical clearance was obtained from the research ethical committee of Department of Medical Laboratory sciences. An informed consent was obtained before collection of nasal swab and results were used in the management of patients. Those patients who clinically diagnosed in pediatric EOPD and EPI willing to participate in the study and able to give nasal swab during the study period was informed about the purpose of the study and written consent were sought for the study. Any information related with the patient and clinical history was kept confidential.

5.10 Dissemination and Utilization of Results

After the completion of the study the research were disseminated to Department of Medical Laboratory Sciences, College of Health Science, and Addis Ababa University. It was also be submitted for scientific publication.

5.11 Operational Definitions

Antimicrobial resistance: occurs when microorganisms change in ways that render the medications used to cure the infections they cause ineffective.

Multidrug resistance (MDR): is antimicrobial resistance shown by a species of microorganism at least to one drug in three different classes of antibiotics.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an infection caused by a type of *Staphylococcus* (staph) bacteria that's resistant to some antibiotics.

6 Results

6.1Characteristics of Study population

A total of 424 mother child were enrolled from March to July 2018 in TASH. Among the study participants 212 (50%) were mothers and 212 (50%) child resulting in an overall ratio of 1:1. From those children 112(52.8) male were participated. The majority of them 1 month-2 years 141(66.5) followed by 2 years-5 years 36(17.0) and up to 1 month 35(16.5) were participated. The mean age of pediatrics participants in this study was 2.0047 ± 0.58 (SD standard deviation) years. From total 212 mother's participants 66.5% were from pediatrics EOPD. The mean age of study mother's was 29.43± 5.405SD (standard deviation).

Table 1: Socio-demographic characteristic of study participants for nasal culture in TASH, 2018.

Variables		Total number (%)	Negative (%)	*S,aureus*(%)
Sex (child)	male	112(52.8)	96(45.25)	16(3.8)
	female	100(47.2)	91(42.95)	9(2.1)
Age	Up to 1 month	35(16.5)	30(14.1)	5(1.2)
	1 month-2 years	141(66.5)	123(58)	18(4.2)
	2 years-5 years	36(17.0)	34(16.1)	2(0.5)
	18-24(mothers)	42(19.8)	36(17)	6(1.4)
	>24	170(80.2)	145(68.4)	25(5.9)

6.3 Magnitude of *S.aureus* and MRSA

Among the total nasal swab processed 13.2% showed *s.aureus*, of which 25(5.9%) were child and 31(7.3%) mothers. From the children the highest *S.aureus* were isolated from 1 month up to 2 years (4.2%), followed by up to 1 month (1.2%) and finally 2 year up to 5 years (0.5%). From 424 total swabs, 6 maternal-neonatal pairs had *S.aureus* carriage. In this study, MRSA were isolated from mothers in pediatrics EOPD 2(3.6%).

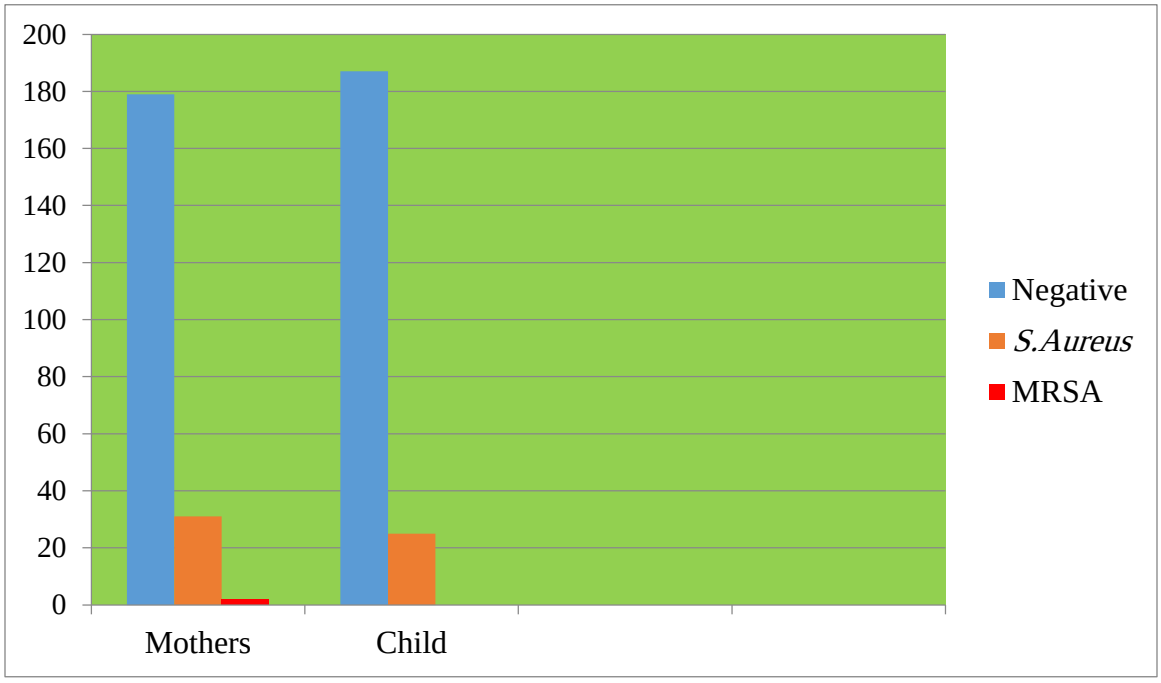

Figure 4. Frequency of *S.aureus* isolated from nasal swab from mother child pair in EPI and pediatrics EOPD TASH, 2018

6.4 Antimicrobial susceptibility Testing

Isolates identified as *S. aureus* were subjected to antimicrobial susceptibility tests using the Kirby Bauer disk diffusion method as described by CLSI. About 89.3% of *S.aureus* was resistance to penicillin and 17.9% which were also resistance to Gentamicin (Tobramycin). All *S.aureus* isolate were sensitive to Clindamycin. On the other hand 98.2% susceptible to Trimethoprim-Sulphamethoxazole and only 7.1% resistant to Erythromycin.

Table 2: Antimicrobial susceptibility patterns of *S. aureus* isolates

Antimicrobial drugs	*Staphylococcus aureus*total isolate (56)	
	Resistant (%)	Susceptible (%)
Cefoxitin (Oxacilin)	2(3.6)	54(96.4)
Penicillin	50(89.3)	6(10.7)
Clindamycin	0(0)	56(100)
Erythromycin	4(7.1)	52(92.9)
Trimethoprim-Sulphamethoxazole	1(1.8)	55(98.2)
Gentamicin	10(17.9)	46(82.1)
Tobramycin	10(17.9)	46(82.1)
Ciprofloxacin	8(14.3)	48(85.7)

6.4.1 Methicillin resistant *Staphylococcus aureus* (MRSA)

Susceptibility test of cefoxitin disk from a total of 56 *Staphylococcus aureus* isolates, showed that 2(3.6%) were resistance to cefoxitin. Both resistances to cefoxitin predicted for oxacillin resistance and other β-lactamase, which includes the penicillins (methicillin, dicloxacillin, nafcillin, etc.) and the cephalosporins generations. Therefore the magnitude of methicillin-resistance *Stapylococcus aureus (*MRSA) was 3.6% of the isolates.

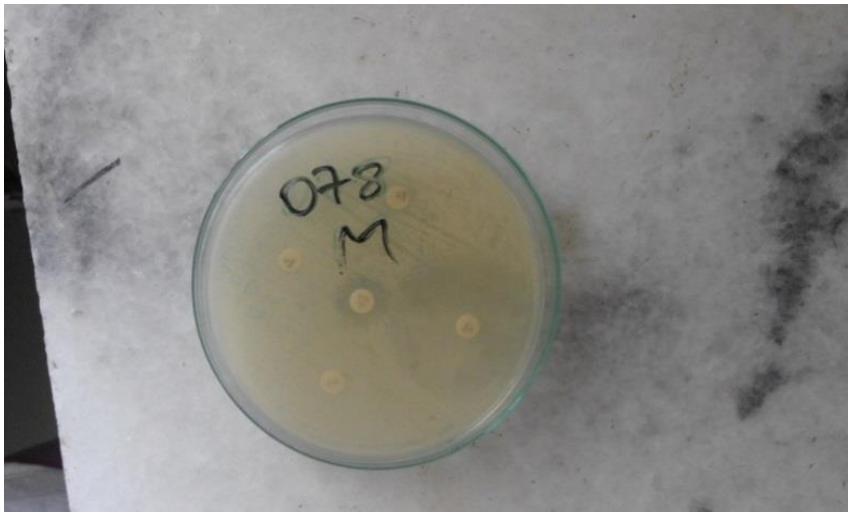

Figure 5: Typical example of Methicillin resistant *Staphylococcus aureus*

6.5 Drug resistance pattern/Anti-biogram of MRSA

Eight (14.3%) of the isolates were multi-drug resistance. According to Magiorakos et al. [52] MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories.A higher number of multi-drug resistances were observed in triple drugs among penicillin, ciprofloxacilin and tobramycin (gentamicin). Two of the isolates were resistance to four drugs, penicillin G, Trimethoprim-sulfamethoxazole, Erythromycin, Cefoxitin and gentamicin (tobramycin).

Table 3: Drug resistance pattern of MRSA isolates for different antibiotics

No of Antibiotics	Drug Resistance pattern	Resistance strains no (%)
2	Pen/Cipro	1(7.14)
	Pen/cefo	1(7.14)
	Ery/cipro	1(7.14)
	Pen/Genta	3(21.4)
3	Ery/Pen/Genta	1(7.14)
	Pen/Ery/Cipro	1(7.14)
	Pen/Genta/Cipro	3(21.4)
4	Pen/Ery/Genta/Cipro	2(14.3)
5	Pen/Ery/SXT/Cefo/Genta	1(7.14)

7. Discussion

Antibiotic resistance continues to be a global setback in the management of common bacterial infections diseases. Particularly, the problem of antibiotic resistance is highly pronounced in resource limited countries, including Ethiopia, where infectious diseases are rampant [53].

S. aureus is a human pathogen which primarily colonizes the nose [54]. In the past few decades, methicillin-resistant strains have come to predominate in both health care-associated and community-associated *S. aureus* infections [55]. However, the epidemiology and microbiology of CA-MRSA and HA-MRSA strains have differed [56]. CA-MRSA has involved younger patients and caused predominantly skin and soft tissue infections, while HA-MRSA has involved older patients who have typical health care-associated risk factors (e.g., indwelling catheter or percutaneous device). However, recent studies describing CA-MRSA strains in the hospital setting suggest that clinical presentation and epidemiologic risk factors are no longer sufficient to reliably define molecular strain types [57-59].

In this study 212 mother child (under 5 year's age) pair were involved from EPI and pediatrics EOPD. The overall prevalence of *S. aureus* and MRSA in this study were 56 (13.2%) and 2 (3.6%). MRSA prevalence (3.6%) is lower than study conducted in Nashville, Tennessee 10% to 17% of mothers, with the highest prevalence at enrollment and Infant MRSA colonization peaked at 2 months of age, with 20.9% of infants colonized [60] and similar to study conducted in Florida USA Only 2.1% of mothers and 0.7% of infants carried MRSA [29].

Another study conducted in USA child care centers overall 210 children (18.1%) were colonized with *S. aureus*. Of these, 195 (16.8% of total) were colonized with MSSA while 15 (1.3% of total) were colonized with MRSA, while compare to our study the carriage was similar but different in isolation technique and only single child are include in the study [61].

In this study *S.aures* and MRSA also lower than research conducted in Dar es Salaam, Tanzania 12 (10.5%) were MRSA. This increased in isolation rate of MRSA could be due to different in isolation technique (pulsed field gel electrophoresis) [30].

In Tripoli-Libya the nasal carriage rate of MRSA among inpatient children (8.3%, 24 of 289), their mothers (11%, 22 of 200).

Our rate of MRSA among child (0%) is lower than a Cohort study conducted in Greece 128 newborn infants and their mother. The carriage rate was 43.6% in infants and 42.5% in mothers .*S. aureus* was isolated in 17.7% and 15.7% swabs from infants and mothers, respectively. Carriage rates were higher in infants with carrier mothers, non-smoking mothers, and many siblings [62].

A study conducted in Gabonese mother–infant pairs was assessed at delivery and after 1, 9 and 12 months. Mother-to-infant transmission was evident in 5.6%. Maternal colonization was a risk factor for S. aureus carriage in infants [26].

According to study conducted in Jimma, the isolation rate of *S.aureus* was 47.3% among school children aged 5-15 years and The variation in the isolation rate of *S.aureus* among different studies could be due to difference in age group and isolation techniques [32].

In Dessie from 118 healthcare workers, 12.7% of all HCWs were identified as MRSA carriers [31].

In a study conducted by Reta A et al found out that the isolation rate of methicillin resistant *staphylococci aureus* (MRSA) was 17(13.8%) [50]. which was slightly higher than our isolation rate. Among HIV Infected Pediatric Patients in Northwest Ethiopia the prevalence of MRSA colonization in this study was 16.8% and this also higher than our study [20].

In our study the resistance pattern of the antibiotics are Cefoxitin 2(3.6), Penicillin 50(89.3), Clindamycin 0(0), Erythromycin 4(7.1), Trimethoprim-Sulphamethoxazole 1(1.8), Gentamicin (Tobramycin) 10(17.9), and Ciprofloxacin 8(14.3).

When compare our study with other study conducted another country lower in isolation rate due to different in isolation technique. Study conducted in Ethiopia such as in Jimma, Dessie, Northwest Ethiopia and the like our study the isolation rate is lower due use of improved infection prevention system, use of anti-biogram and use empirical treatment.

8. Strength and limitation of the study

Strength

- Show the magnitude and antimicrobial resistance pattern of *S.aureus* among mother child pair under five years of age first time in TASH.
- Point out last line drugs resistance antimicrobials.

Limitation

- Even though our study identifies the magnitude of *S.aures* and MRSA from mother and child pair, we could not able to characterize the isolate further.

9. Conclusions and Recommendation

Conclusions

Although identification and prevention techniques have improved, *S.aureus* remains a major healthcare issue. MRSA bacteremia can be challenging to manage, especially in patients at high risk of complications or in those with toxigenic or multidrug-resistant strains. Early identification of MRSA is an important step toward timely implementation of appropriate treatment.

This study showed lower rate of nasal carriage of mother child pair of *S.aureus* compare to other study conducted in Ethiopia. Therefore developing decolonization protocols and proper utilization of drugs are needed to reduce the transmission of *S.aureus* and the burden of MRSA.

Recommendation

Based on the finding of our study, we recommend that

- Use antibiotics only when prescribed by a doctor, completing the full prescription, even if they feel better and never sharing antibiotics with others or using leftover prescriptions.
- Clinicians should avoid prescribing last line antibiotics for any patients and updated guideline for prescribing drugs to address drug resistance.
- Nasal screening for MRSA in patients and in the units should be mandatory for effective infection control and prevention.
- Include more study groups.
- Various effective methods of infection prevention control should be utilized to help reduce the incidence of *S.Aureus* and MRSA infections.
- A prevalence study of nasal carriage of *S.aureus* among health care providers in these units should be conducted to find out if they are not sources of MRSA transmission.

10. References

1. Baron S, Baron EJ, Aly R, Albrecht T, Baxby D, Becker Y et al. Medical Microbiology 4th edition. University of Texas Medical Branch at Galveston 1996.
2. Klein E, Smith DL, Laxminarayan R. Hospitalizations and Deaths Caused by Methicillin-Resistant *Staphylococcus aureus*, 1999–2005.Emerging Infectious Disease. 2007;13:12:1840-46.
3. Noorani HZ, Adams E, Glick S, Weber S, Belinson S, Aranson N. Screening for Methicillin-Resistant *Staphylococcus aureus*: Future Research Needs. Agency for Healthcare Research and Quality (US).2013:13-EHC056-EF.
4. Zervou FN, Zacharioudakis IM, Ziakas PD, Mylonakis E. MRSA Colonization and Risk of Infection in the Neonatal and Pediatric ICU: A meta-analysis 2014:133; 4.
5. Cardoso JL, Lencastre H, Kipnis A, Pimenta FC, Luciana SC, Oliveira et.al. Molecular Epidemiology and Risk Factors for Nasal Carriage of *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* in Infants Attending Day Care Centers in Brazil. Clinical Micro J. 2009:47; 12: 3991–97.
6. David MZ, Daum R. Community-Associated Methicillin-Resistant *Staphylococcus aureus:* Epidemiology and Clinical Consequences of an Emerging Epidemic. Clinical Micro J. 2010:23; 3: 616-87.
7. Nelson MU, Gallagher PG. Methicillin-Resistant *Staphylococcus aureus* in the Neonatal Intensive Care Unit. Peri.2012:36; 6:424-30
8. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks Clin Microbiol. 1997; 10(3):505-20.
9. Gorwitz RJ, Kruszon-Moran D, McAllister SK, McQuillan G, McDougal LK, Fosheim GE, et al, Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. J Infect Dis. 2008; 197(9):1226-34.
10. Parvizi J, Goyal N Orthopedics today, November 2010. Available https://www.healio.com/orthopedics/hip.
11. Chambers HF and Deleo FR. Waves of Resistance: *Staphylococcus aureus* in the Antibiotic Era. Nat Rev Microbiol. 2009; 7(9): 629–641.

12. Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA, and Rood JI 2000. Mechanisms of resistance to β-lactam antibiotics in Gram-positive pathogens. American Society for Microbiology 2000. Washington, DC, USA. 609–620.
13. Chambers HF. Methicillin resistance in *staphylococci*: molecular and biochemical basis and clinical implications. Clin Microbiol Rev. 1997 Oct; 10(4):781-91.)
14. Lim D, Strynadka NC. Structural basis for the beta lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. J Clin Invest. 2003; 111(9): 1265–1273.
15. Truque NJ, Tedeschi S, Saye EJ , McKenna BD , Langdon W, Wright JP et al. Relationship between Maternal and Neonatal *Staphylococcus aureus* Colonization.2012:129;5:1252–59.
16. World Health Organization: Antimicrobial resistance a global threat. Drug Ther Bull. 2014; 52(7):77.
17. Fischetti VA, Novick RP, Ferretti JJ, Portnoy DA, and Rood JI 2000. Mechanisms of resistance to β-lactam antibiotics in Gram-positive pathogens. American Society for Microbiology 2000. Washington, DC, USA. 609–620.
18. Stefani S, Chung RD, Lindsay AJ, Friedrich WA, Kearns MA and Westh H. et al. Meticillin-resistant *Staphylococcus aureus* global epidemiology and harmonization of typing methods. International Journal of Antimicrobial Agents 2012: 39:4:273-282.
19. Birgitte K, Giersinga Sana S, Kayvon D and Vasee Moorthya M Status of vaccine research and development of vaccines for *Staphylococcus aureus*. Journal of vaccine 2016:34; 26:2962-2966.
20. Lemma TM, Zenebe Y, Tulu B, Mekonnen D, Mekonnen Z. Methicillin Resistant *Staphylococcus aureus* among HIV Infected Pediatric Patients in Northwest Ethiopia: Carriage Rates and Antibiotic Co-Resistance Profiles PLoS One. 2015; 10:9: e0137254.
21. Herriman R. Characteristics of *Staphylococcus Aureus* by Last Updated: Aug 14, 2017.
22. Wertheim HF, Melles D , Vos MC, van Leeuwen W, van Belkum A and Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Journal of Lancet Infectious Disease 2005:5; 12:751-62.
23. Egyir B, Guardabassi L, Esson J, Nielsen SS, Jemima Newman M and Kwasi Addo K et al. Insights into Nasal Carriage of *Staphylococcus aureus* in an Urban and a Rural Community in Ghana.PLoS ONE 2014: 9; 4: e96119.

24. Chatzakis E, Scoulica N, Papageorgiou S, Maraki G, Samonis et al. Infant colonization by Role of maternal carriage. Micro J2011; 30 :(9):1111-1117.
25. Lin J, Wu C, Yan C, Ou Q, Lin D, Zhou J, Ye X, Yao Z. A prospective cohort study of Staphylococcus aureus and methicillin-resistant *Staphylococcus aureus* carriage in neonates: the role of maternal carriage and phenotypic and molecular characteristics.
26. Panel F, Schaumburgab S, Alabibc G, Mombo-Ngomacde H, Kababc R, Zolekobce D, Diopbc R. Transmission of *Staphylococcus aureus* between mothers and infants in an African setting.
27. Wachter KInfant *S. aureus* Colonization Rises after Birth in Cases of Maternal Nasal Carriage 2008.
28. Rijal KR, Pahari N, Shrestha BK, Nepal AK, Paudel B, Mahato P et al. Prevalence of methicillin resistant *Staphylococcus aureus* in school children of Pokhara Nepal. Med J. 2008:10; 3:192-5.
29. Reusch M, Ghosh P, Ham C, Klotchko A, Singapuri S, Everett G. Prevalence of MRSA colonization in peri-partum mothers and their newborn Infants. Scand J Infect Dis. 2008; 40(8):667-71.
30. John MS, Said A, Bjorn B, Namala M , Mabula K , and Karim M et al. Microbial Drug Resistance. High Nasal Carriage of Methicillin-Resistant *Staphylococcus aureus* Among Healthy Tanzanian Under-5 Children 2014:20; 1: 82-88.
31. Shibabaw A, Abebe T, Mihret T. Nasal carriage rate of methicillin resistant *Staphylococcus aureus* among Dessie Referral Hospital Health Care Workers; Dessie, Northeast Ethiopia. Antimicrobial resistance and infection control. 2013:2; 25.
32. Kejela T, Bacha K. Prevalence and antibiotic susceptibility pattern of methicillin-resistant *Staphylococcus aureus* among primary school children and prisoners in Jimma Town, Southwest Ethiopia. Micro J.2013: 12; 11.
33. Ahmad S, Mustafa MM, Shamweel H. Methicillin Resistant *Staphylococcus aureus* a Challenge for Health Care- Professionals and Patients. Acta Scientific Pharmaceutical Sciences 2017:1; 2: 79-88.
34. Jimenez-Truque N. Infectious Diseases in Children.2012:2011-2308.
35. Gordon JR, Lowy DF. Pathogenesis of Methicillin-Resistant *Staphylococcus aureus* Infection Clinical Infectious Disease. 2008: 1; 46:S350–S359.

36. Stephen H. Gillespie, Kathleen B. Bamford. Medical Microbiology and Infection at a Glance, Fourth Edition. 2012:33.
37. Kobayashi DS, Malachowa N, DeLeo FR. Pathogenesis of *Staphylococcus aureus* Abscesses.Patho J. 2015:185; 6: 1518–1527.
38. DeLeo FR, An Diep B, Otto M. Host Defense and Pathogenesis in *Staphylococcus aureus* Infections. Infectious Disease clinics of North America 2009:23; 1:17-3.
39. Todar K online textbook of bacteriology Avaliable http://text book of bacteriology .net/*s.aureus*.(Assessed 10/10/2017).
40. Francois P, and Schrenzel J. Rapid Diagnosis and Typing of *Staphylococcus aureus.* Molecular Genetics. Caister Academic Press.2008 ISBN 978-1-904455-29-5.
41. Mackay IM 2007. Real-Time PCR in Microbiology: From Diagnosis to Characterization. Caister Academic Press. ISBN 978-1-904455-18-9 United Kingdom 2007.
42. Seiken, Denka. "MRSA latex test for PBP2" Available https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/MRSALatexTest.htm.
43. Les Moore M. Staph-Infection-Resources. Available https://www.staph-infection-resources.com.
44. Lamy Filho F, Cavalcante de Sousa SH, Sousa Freitas IJ, Carvalho Lamy Z, Ferreira Simões VM, Moura da Silva AA et al. Effect of maternal skin-to-skin contact on decolonization of Methicillin-Oxacillin-Resistant *Staphylococcus* in neonatal intensive care units. BMC J. 2015: 15; 63.
45. Gorwitz JR, Jernigan BD, Powers HJ, Jernigan AJ. Centers for Disease Control and Prevention Strategies for Clinical Management of MRSA in the Community: 2006.
46. Soltani B, Ardakani AT, Moravveji A, Erami M, Rezaei MH, Moniri R. Risk Factors for Methicillin Resistant *Staphylococcus aureus* Nasal Colonization of Healthy Children. Journal of Micro J. 2014:7; 9
47. Bope TE, D.Kellerman R. conn's current therapy 2015.
48. Ward S, Hisley S. Maternal child nursing care optimizing out comes for mothers, children, and families. 2nd edition.
49. http://www.aau.edu.et/services/hospital/background-of-college-of-health-sciences/

50. Reta A, Gedefaw L, Sewunet T andBeyene G. Nasal carriage, risk factors and antimicrobial susceptibility pattern of methicillin resistant *staphylococcus aureus* among school children in Ethiopia. Journal of Medical Microbiology & Diagnosis 2015: 4; 177.
51. Clinical and laboratory standards institute performance standards for antimicrobials susceptibility testing M100 27 th 2016
52. Magiorakos PA, Srinivasan A, Carey BR, Carmeli Y, Falagas EM, Giske GC, et al. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria. Microbiol J.2012; 18:268–81.
53. Eshetie S, Tarekegn F, Moges F, Amsalu A, Birhan W, Huruy K. Methicillin resistant *Staphylococcus aureus* in Ethiopia: a meta-analysis.
54. Lowy F. D. *Staphylococcus aureus* infections. N. Engl. J. Med1998:339:520–532
55. Woodford N, Livermore M. Infections caused by Gram-positive bacteria: a review of the global challenge. 2009 J. Infect. 59(1):S4–S16.
56. Diederen M., Kluytmans J. A. 2006. The emergence of infections with community-associated methicillin resistant *Staphylococcus aureus*. J. Infect. 52:157–168.
57. Milstone AM, Song X, Beers C, Berkowitz I, Carroll KC, Perl TM. Unrecognized burden of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococcus carriage in the pediatric intensive care unit. Hosp Epidemiol J 2008; 29:1174–6.
58. Khoury J, Jones M, Grim A, Dunne WM Jr, Fraser V. Eradication of methicillin-resistant *Staphylococcus aureus* from a neonatal intensive care unit by active surveillance and aggressive infection control measures. Infect Control Hosp Epidemio. 2005; 26:616–21.
59. Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med. 2006; 355:666–74.
60. Tedeschi S, Saye E, McKenna B, Langdon W , Wright J, Alsentzer A et al. Relationship between Maternal and Neonatal *Staphylococcus aureus* Colonization 2012; 129(5): 1252–1259.
61. Miller M, Weber D, Goodrich J, Popo witch E, Poe M, Nyugen V et al. Prevalence and Risk Factor Analysis for Methicillin-Resistant *Staphylococcus aureus* Nasal Colonization in Children Attending Child Care Centers.

62. Chatzakis E, Scoulica N, Papageorgiou S, Maraki G, Samonis, et al. Infant colonization by Role of maternal carriage. Micro J2011; 30 :(9):1111-1117.

Annexes

ANNX I: Information sheet

Study title: Magnitude and Antimicrobial Resistance Pattern of *S.aureus* among Mother Child pairs isolated from nasal swab at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Principal investigator: Mekedlawit Abtew

Dear participant

I am learning in Addis Ababa University College of health science Department of Medical Laboratory Sciences, School of Allied Health Science. I would like to interview you few questions about blood stream infection for the cause of febrile illness.

Advisor: Kassu Desta

Ethical approval and confidentiality: The ethical clearance was obtained from the research ethical committee of Department of Medical Laboratory sciences. I assure you all the information regarding to the result being confidential. Your participation is voluntary and you are not obligate to answer any questions and give any specimens that you are not volunteer to answer. I would like to appreciate if you give me a few minutes to answer my questions but if you are not comfortable feel free to stop it any time.

Are you volunteer to continue? If yes, continue to next page for interview. If no, continue next participant

Introduction: *S.aureus* is a leading cause of hospital acquired infections. It's the primary cause of lower respiratory tract infections, surgical site infections, second leading cause of nosocomial bacteremia and cardiovascular infection. MRSA infections in the pediatric population range from skin and soft tissue to lower respiratory, bloodstream infections and are associated with significant morbidity and hospital costs. Neonates are exposed to Staphylococcus aureus shortly after birth and can become colonized quickly after contact with adult skin or their environment.

Purpose of the study: this study is to isolate mother and child pairs of *Staphylococcus aureus* isolated from nasal swab and their antimicrobial resistance pattern. You are kindly requested to be included in the study which have importance for management of your and child treatment and

your cooperation and willingness for about 15 minute of interview and providing specimen will be very helpful in identifying the problems related to the issue.

The specimen that you and your baby will provide a nasal sample within short time. Giving to these specimens does not affect your health.

Role of principal investigator. Culture the specimen and isolate the bacteria. In addition, antimicrobial susceptibility testing were carried out in microbiology laboratory

Thank you for your cooperation!!!

Annex II: Consent Form

I have read the information sheet concerning this study (or have understood the verbal explanation) and I understand what will be required of me and what will happen to me if I take part in it. I also understand that any time I may withdraw from this study without giving a reason whenever discomfort me and my child.

May I continue the interview?

Yes ☐ Continue the interview

No ☐ Stop the interview and thank the respondent

Witness's signature certifying that the informed consent has been given

Witness's signature _____ Date _____

Introduction to the Interview

Thank you for deciding to participate in the interview and for coming to this session. Previously (on the statement of consent form), we have discussed briefly on the purpose of the research, how you were identified, and your part in the research study. Now I am going to have discussion with you on the relevant topic items. Before going to the discussion, would you tell me important backgrounds such as age, educational background etc.? There is no right or wrong answers. All answers /responses/ ideas you provide are equally important and you are requested to respond honestly from your experiences and beliefs. I may interrupt and probe your ideas. Once again I would like to tell you that what we are going to discuss is very confidential and it will be used only for the research.

Thank you!

Annex III: Questionnaire

Addis Ababa University College of health science, Department of Medical Laboratory Sciences.

Questionnaire A child questionnaire formats

1. Date ----------
2. Code No ----------
3. Address: sub-city---------- Woreda----------
4. Tele ----------

Supervisor's name: ---------- Signature ----------

Time started: ---------- Time finished: ----------

101. Pediatric Age ---------- years

102. Medical registration number (MNR) ----------

103. Sex: □ male □ female

104. Mother's educational status: a) illustrate □ b) read and write □ c) elementary school □ d) high school □ e) collage/university □

105. Family size ----------

106. Family monthly income----------birr/ETB

107. Current unit of diagnosis; EPI □ pediatric OPD □

108. Breast feeding yes □ no □

109. Earlier antibiotic use within 2 weeks □ within 2–4weeks □ within 1–6 months □ Within 6–12 months □ no □

110. Is the child sleep with the parent? Yes □ No□

111. Does the child have history of hospital admission? Yes □ No□

112. Does the pediatric have history of the following conditions?

A. Renal and/or liver disease □ Yes □ no

B. Intravascular catheters □ yes □ no

C. Hemodialysis □ yes □ no

D. Intravenous drug abusers □ yes □ no

113. Do you have Family member work in health care? Yes□ no□

114. Do you have Family member work in child care? Yes □ no □

115. Do you have contact with Pet animal? Usually □ sometimes □ not at all □

116. Immunization Fully immunized □ Up-to-date □ Default □ not immunized □

Questionnaire B Mother Questionnaire formats

1. Date ----------
2. Code No ----------
3. Address: sub-city---------- Woreda----------
4. Tele ----------

Supervisor's name: ---------- Signature ----------

Time started: ---------- Time finished: ----------

101. Mother Age ---------- years

102. Medical registration number (MNR) ----------

103. Educational status: a) illustrate□ b) read and write□ c) elementary school □
d) high school □ e) collage/university □

104. Family size ----------

105. Family monthly income----------birr/ETB

106. Breast feeding yes □ no □

107. Earlier antibiotic use within 2 weeks □ within 2–4weeks □ within 1–6 months □
Within 6–12 months □ no □

If taken, Please specify the antibiotics ----------

108. Do you sleep with your child? Yes □ No□

109. Do you have history of the following conditions?

A. Renal and/or liver disease □ Yes □ no

B. Intravascular catheters □ yes □ no

C. Hemodialysis □ yes □ no

D. Intravenous drug abusers □ yes □ no

110. Do you or other Family member work in health care? Yes□ no□

111. Do you or other Family member work in child care? Yes □ no □

112. Do you have contact with Pet animal? Usually □ sometimes □ not at all □

Thank you!

Annex IV: Amharic version of participant Information Sheet, consent and incent form

:ስለጥናቱ ማስተዋወቂያ በጥናቱ ለመሳተፍ ፈቃደኝነት መጠየቂያ የአማርኛ ቅጽ ከአምስት አመት በታች ለሆኑ ህፃናት እንዲሁም በእናቶቻቸዉ ላይ ስለ እስታፋይሎኮከስ አዉረስ እና የመድሀኒት መለማመድ ላይ የሚደረግ ጥናት ነዉ።።

<u>ስለ ጥናቱ ማስተዋወቂያ ቅፅ</u>

ጤና ይስጥልኝ? እኔ ስሜ መቅደላዊት አብተዉ እባላለዉ።።የምማረው በአዲስ አበባ ዩኒቨርሲቲ ህክምና ትምህርት ቤት የሜዲካል ላቦራቶሪ ተማሪ ስሆን፤ እርስዎን ቃለመጠይቅ ለመጠየቅ የፈለግኩት ስለ እስታፋይሎኮኮ ስአዉረስ ጥናት ለመስራት ሲሆን፤ የጥናቱ ዓላማ ከአምስት አመት በታች በሆኑ ህፃናት እንዲሁም በእናቶቻቸዉ ላይ ስለ እስታፋይሎኮኮስ አዉረስ ህክምና እና በሚታዘዙ ፀረተህዋሥያን የፈዋሽነት ደረጃ ግንዛቤ ለማገኘት ይረዳል።።እርሰዎንም በዚህ ጥናት እንዲሳተፉ በትህትና እንጠይቀዎታለን።።በዚህ ጥናት በመሳተፈዎ የምናገኘው መረጃ ለጥናታችን ውጤታማነት እነዲሁም በጥናቱ ውጤት ላይ ከፍተኛ አስተዋፅዎ ይኖረዋል።።ናሙና ሲወሰድ በልጅዎ እንዲሁም በእርሶም ጤንነት ላይ ምንም አይነት የጤና ችግር አያስከትልም።።በጥናቱ ውስጥም የሚሠጡት መረጃ ሙሉ በሙሉ ሚስጢራዊነቱ የተጠበቀ ነው።። ስለዚህም በዚህ ቃለ-መጠይቅ በመሳተፈዎ ምስጋናዬ የላቀ ነው።።ስለዚህ በጥናቱ ለመሳተፍ የእርሥዎ ሙሉ ፈቃድ አስፈላጊ ነው።።በተጨማሪም ለመመለስ የማይፈልጓቸው ጥያቄዎች ካሉ ጥያቄዎችን ለመመለስ አይገደዱም።።አንዲሁም በጥናቱ ላለመሳተፍ ከፈለጉ በማንኛውም ጊዜ ማቋረጥ ይችላሉ።።ቃለ መጠየቁን ለማካሄድ እስከ20 ደቂቃ ይወስዳል።።

መጠይቁን እንድቀጥል ፈቃደኛ ነዎት

1.አዎ ፈቃደኛ ነኝ	☐	ቃለ መጠይቁ ይቀጥላል
2. አይ ፈቃደኛ አደለሁም	☐	ቃለ መጠይቁን በማቆም አመስግነው ይለያዩ

ቃለ መጠየቁን በተመለከተ ወይም አጠቃላይ ስለ ጥናቱ ማንኛውንም አይነት ጥያቄና አስተያየት ቢኖረዎት በሚከተሉት አድራሻዎች መጠቀም ይችላሉ።።

አድራሻ፡ አዲስ አበባ ዩኒቨርቲ ህክምና ትምህርት ቤት የሜዲካል ላቦራቶሪ ት/ት ክፍል

መቅደላዊት አብተዉ፤ ስልክቁጥር፡ +251 911756156

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ከመጠየቁ በፊት የተጠያቂውን ስምምነት ማረጋገጫ ቅፅ

ከላይ በመግቢያው ላይ የተጠቀሰውን መረጃ አንብቢያለሁ ወይም በቃል የተሰጠኝን ማብራሪያ ተረድቻለሁ፡፡በዚህ መሰረት ከእኔ የሚጠበቅብኝን ድርሻ በሚገባ አውቄያለሁ እና በዚህ ጥናት ላይ በመሳተፌ ሊከሰቱ የሚችሉትን ሁኔታዎች ተገንዝቢያለሁ፡፡ጥናቱን በማንኛውም ሠዓት ያለምንም ቅድመ ሁኔታና ምክንያት እራሴን ከተሳታፊነት የማግለል ሙሉ መብት እንዳለኝ ተረድቻለሁ፡ ይህን ውሳኔዬን ተከትሎ በእኔም ሆነ በልጇ ላይ በምንፈልገው የጤና አገልግሎት ላይ ምንም አይነት አሉታዊ ተጽዕኖ እንደማይደርስብኝ ተረድቻለሁ፡፡

ፊርማ ---------- ቀን ----------

ጥናቱን በተመለከተ የቃል ማብራሪያ የተሰጠ መሆኑን የሚያረጋግጠው የቃለ መጠይቁ አድራጊ ስምና ፊርማ

የጠያቂው ስም----------

ፊርማ----------

ቀን----------

መጠይቁን እንድቀጥል ፈቃደኛ ኖት?

1.አዎ ፈቃደኛ ነኝ ☐ ቃለ መጠይቁ ይቀጥላል

2. አይ ፈቃደኛ አደለሁም ☐ ቃለ መጠይቁን በማቆም አመስግነው ይለያዩ

የመጠየቁ ውጤት መግለጫ

ሀ.ሙሉ በሙሉ የተሞላ ☐ ለ. በከፊል የተሞላ ☐ ሐ.ተጠያቂው ፈቃደኛ አይደለም ☐

መ. ሌላ ካለ----------

መጠየቁን የሞላው ሰው ስም----------ፊርማ--------- ቀን----------

በአማርኛ የተዘጋጀ የህፃኑ/• መጠይቅ

በአዲስ አበባ ዩኒቨርሲቲ ህክምና ትምህርት ቤት የሜዲካል ላቦራቶሪ ት/ት ክፍል፡፡

1. ቀን ----------
2. መ.ቁጥር ----------
3. አድራሻ..፡ ----------ክ/ከተማ፡---------- ወረዳ፡----------
4. ስ.ቁ. ----------

የሱፐርቫይዘሩ ስም---------- ፊርማ----------

የተጀመረበት ሰአት ---------- ያለቀበት ሰአት -------

101. የህፃኑ/• እድሜ፡----------
102. ፆታ፡ ወንድ□ ሴት□
103. የካርድ ቁጥር፡----------
104. የወላጅ የትምህርት ደረጃ፡ ማንበብና መጻፍ የማትችል □ ማንበብና መጻፍ የምትችል □
የመጀመሪያ ደረጃ □ ሁለተኛ ደረጃ □ ኮለጅ /ዩኒቨርሲቲ □
105. የቤተሰብ ብዛት፡ ----------
106. የቤተሰቡ ወርሀዊ ገቢ፡----------
107. አገልግሎት የሚያገኙበት ክፍል ፡ ክትባት□ ከ 5 አመት በታች ህክምና □
108. የእናት ጡት ወተት ፡ ጠብቷል □ አልጠባም □
109.በቅርቡ ፀረ-ባክቴሪ ያመድሀኒት ወስዷል? በዚህ ሁለት ሳምንት□ ከሁለት ሳምንት - አራት ሳምንት□
ከአንድ ወር - ስድስት ወር□ ስድስት ወር - አንድ አመት□ የለም□
110. ህፃኑ የሚተኛው ከቤተሰብ ጋር ነው? አዎን □ አይደለም□
111. ህፃኑ/• ከዚህ በታች ያሉትን ህመሞች ታሞ/ታክሞያዉቃል?

የኩላሊት/ጉበት	አዎ □	አያዉቅም □
የኩላሊትእጥበት	አዎ □	አያዉቅም □
በደም ስር የሚሰጡ መድሀኒት	አዎ □	አያዉቅም □
ካቴተር	አዎ □	አያዉቅም □

112. ከቤተሰቡ መካከል በጤና ተቀም የሚሰራ አለ? አዎ □ የለም □
113. ከቤተሰቡ መካከል ህፃናት ማቆያ የሚሰራ አለ? አዎ □ የለም □
114. ህፃኑ ከቤት እንስሳ ጋር ቀረቤታ አለዉ? አዎ□ ሁልጊዜ□ አልፎአልፎ□ የለዉም□

115. የክትባት ሁኔታ፡

ክትባት የጨረሰ	☐
በመከታተል ላይ ያለ	☐
ክትባት ያቋረጠ	☐
ክትባት ያልወሰደ	☐

የእናቶች መጠይቅ

1. ቀን፡ ----------
2. መ.ቁጥር፡ ----------
3. አድራሻ..፡ ----------ክ/ከተማ፡---------- ወረዳ፡----------
4. ስ.ቁ፡ ----------

የሱፐርቫይዘሩ ስም---------- ፊርማ----------

የተጀመረበት ሰዓት ---------- ያለቀበት ሰዓት ----------

101. እድሜ፡----------

102. የካርድ ቁጥር፡----------

103. የትምህርት ደረጃ፡ ማንበብና መጻፍ የማትችል □ ማንበብና መጻፍ የምትችል □

የመጀመሪያ ደረጃ □ ሁለተኛ ደረጃ □ ኮለጅ /ዩኒቨርሲቲ □

104. የቤተሰብ ብዛት፡----------

105. የቤተሰቡ ወርሀዊ ገቢ፡----------

106.አገልግሎት የሚያገኙበት ክፍል ፡ ክትባት□ ከ 5 አመት በታች ህክምና□

107.ጡት ያጠባሉ? አጠባለዉ □ አላጠባም □

108.በቅርቡ ፀረ-ባክቴሪያ መድሀኒት ወስደዋል? በዚህ ሁለት ሳምንት□ ከሁለት ሳምንት - አራት ሳምንት□

ከአንድ ወር - ስድስት ወር□ ስድስት ወር - አንድ አመት □ የለም□

109. ከልጅ ጋር ነው የሚተኙት? አዎን □ አይደለም □

110. ከዚህ በታች ያሉትን ህመሞች ታመዉ/ታክመዉያዉቃል?

የኩላሊት/ጉበት	አዎ □	አያዉቅም □
የኩላሊትእጥበት	አዎ □	አያዉቅም □
በደም ስር የሚሰጡ መድሀኒት	አዎ □	አያዉቅም □
ካቴተር	አዎ □	አያዉቅም □

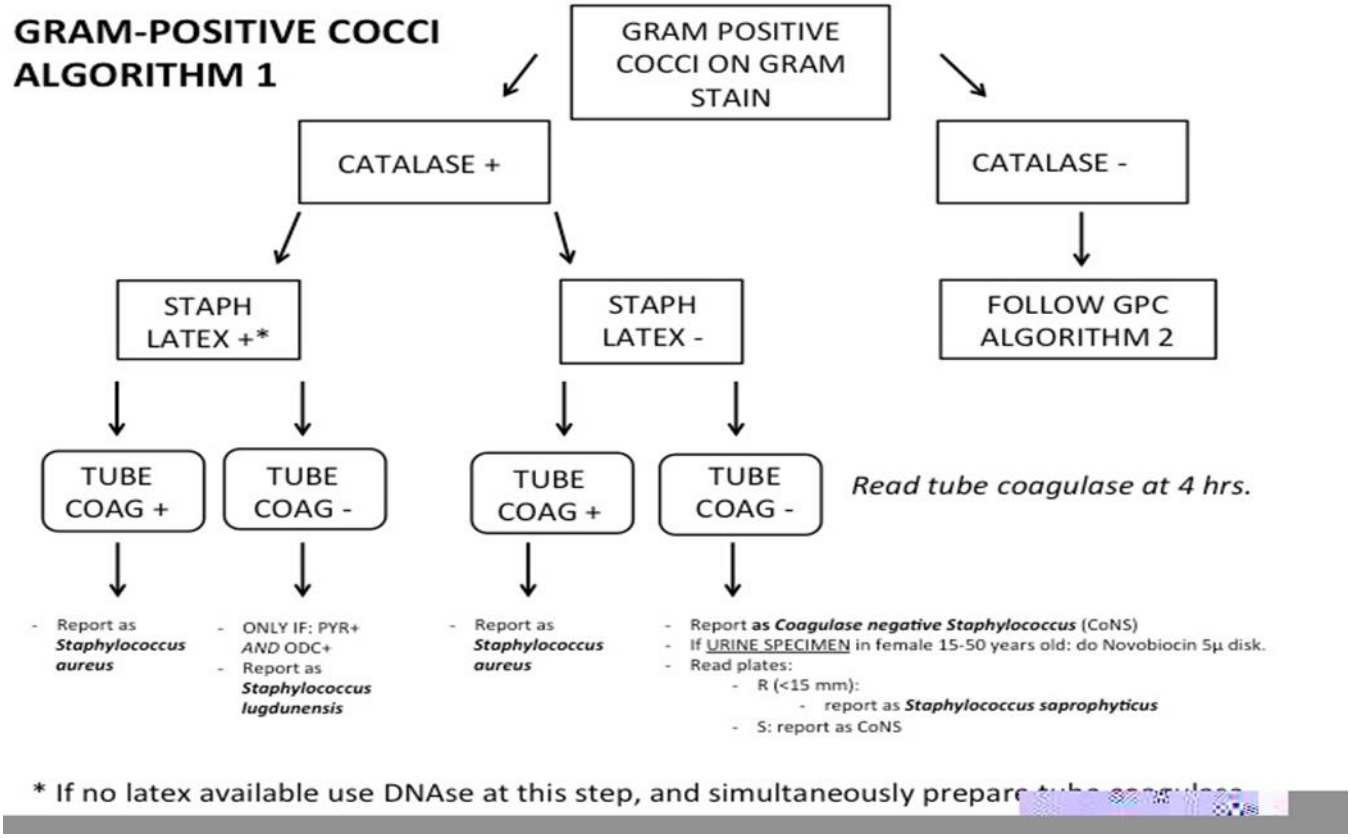
111. እርሶ ወይም ከቤተሰቦ መካከል በጤና ተቐም የሚሰራ አለ? አዎ □ የለም □

112. እርሶ ወይም ከቤተሰቦ መካከል ህፃናት ማቆያ የሚሰራ አለ? አዎ □ የለም □

11. ከቤት እንስሳ ጋር ቀረቤታ አሎት? አዎ□ ሁልጊዜ□ አልፎአልፎ□ የለዉም□

ከልብ አመሰግናለዉ።።

Annex V: Gram Positive Cocci



Annex VI: Zone Diameter Interpretive Standards for Staphylococcus spp

Testing conditions

Media: Mueller-Hinton agar.

Use maximum 12 disks on a 150 mm plate;

Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center.

Inoculum: direct colony suspension equivalent to 0.5 McFarland standards

Incubation: 33-35oc (testing at above 37oc may not detect MRSA), ambient air.

Incubation time: 16-18 hr EXCEPT Coagulase-negative staphylococci and cefoxitin: 24 hours.

When test? #	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A (NB! only S.aureus)	Penicillin G (only S. aureus)	10 unit	≤28	-	≥ 29 NB□	If ≥ 29 mm examine zone edge: report as R if sharp (“cliff”); report as S if fuzzy (“beach”) Always R if cefoxitin R.
A	Cefoxitin (For S.aureus or S.lugdunensis)	30 µg	≤21	-	≥22	Do not report; report oxacillin and other beta lactams based on cefoxitin result (see comments below)
A	Cefoxitin (For CoNS except S.lugdunensis)	30 µg	≤24	-	≥25	Do not report; report oxacillin and other beta lactams based on cefoxitin result (see comments below)
A	Erythromycin (PO only)	15 µg	≤13	14-22	≥23	DO NOT TEST IN URINE OR CSF ISOLATES
A	Clindamycin (PO only)	2 µg	≤14	15-20	≥ 21 NB	DO NOT TEST IN URINE OR CSF ISOLATES Place erythromycin disc and clindamycin disc 12-20mm apart (edge to edge). Report clindamycin as R if “D-phenomenon” is seen (inducible clindamycin resistance) (EUCAST)
A	Trimethoprim-Sulfamethoxazole	1.25/23.75 µg	≤ 10	11-15	≥ 16	
AU	Nitrofurantoin (PO only)	300 µg	≤ 14	15-16	≥ 17	Urine isolates only

	Ampicilin / Amoxicillin	DO NOT TEST				Report as S /R based on Penicillin G result. Always R if cefoxitin R.
	Oxacillin (Cloxacillin available PO and IV)	DO NOT TEST				Report as S /R based on Cefoxitin result
	Amoxicillin+clavulanic acid (PO only)	DO NOT TEST				Report as S /R based on Cefoxitin result
	Cephalotin	DO NOT TEST				Report as S /R based on Cefoxitin result
	Cefuroxime	DO NOT TEST				Report as S /R based on Cefoxitin result
	Cefotaxim	DO NOT TEST				Report as S /R based on Cefoxitin result
	Ceftriaxone	DO NOT TEST				Report as S /R based on Cefoxitin result
	Ceftazidim	DO NOT TEST				Report as R if requested
1	Gentamicin	10 µg	≤ 12	13-14	≥ 15	DO NOT TEST IN CSF UNLESS REQUESTED
3	Tobramycin	10 µg	≤ 12	13-14	≥ 15	DO NOT TEST IN CSF UNLESS REQUESTED
2	Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21	
5	Doxycycline (PO only)	30 µg	≤ 12	13-15	≥ 16	
6	Azithromycin	15 µg	≤13	14-17	≥18	DO NOT TEST IN URINE ISOLATES
7	Trimethoprim (PO only)	5 µg	≤ 10	11-15	≥ 16	Urine isolates only
4	Chloramphenicol	30 µg	≤ 12	13-17	≥ 18	DO NOT TEST IN URINE. ALWAYS TEST IN CSF.

Annex VII: Declaration

The undersigned declares that this proposal complies with the regulations of the University and meets the accepted standards with respect to originality and quality. PI also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. candidate: Mekedlawit Abtew

Signature: _____

Date of submission: _____

This proposal has been submitted with our approval as advisor.

Advisor: Kassu Desta

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.